Project ID/Title: RIGS16-176-0340/ THE STUDIES ON ENVIRONMENTALLY SAFE BIOPOLYMER DERIVED FROM NATURAL BASED ALGAE.

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Author Name(s): ASSOC. PROF. DR. MAIZATULNISA OTHMAN, ASSOC. PROF. DR. SOAUD, DR. AYUNI JAMAL

Department/Kulliyyah/Institute/Centre: KOE/MME

Abstract: In the efforts to sustain the environment, biodegradable plastic has become a great alternative to replace conventional plastic. Hence, this study focuses on a potential biodegradable plastic. In the current study, the properties of algae (matrix) were investigated by adding acetic acid and cinnamon powder as filler and antimicrobial agent. The amount of acetic acid varies from 0.1, 0.3 and 0.5%, while, the cinnamon content between 1, 3 and 5%. The film was fabricated using the solution casting method. Upon fabrication, the physical and mechanical properties of the films were characterized using tensile test, Fourier Transform Infrared Spectrometry (FTIR) analysis, Scanning Electron Microscopy (SEM) analysis and biodegradation test. Based on the results, the addition of acetic acid and cinnamon are capable of affecting the tensile property of the algae film. Initially, it indicated that the acetic acid reduced the tensile property and affected the elongation at break of the algae film. However, the tensile strength of the film was altered by adding a certain amount of cinnamon. The maximum tensile strength was achieved by the addition of 5% cinnamon which exhibited a good intermolecular interaction between the algae and cinnamon molecules. The tensile strength which was measured at 4.80 MPa correlated with the morphological structure. The latter was performed using SEM, where, the surface showed the absence of a separating phase between the algae and cinnamon blend. Moreover, the addition of acetic acid into the algae film clearly indicated that the acetic acid molecules affect the adjacent molecules by increasing the distance and reducing the internal force giving more flexibility to the film. This was evidenced by the Fourier Transform Infrared (FTIR) analysis which confirmed the occurrence of no chemical reaction between the algae and acetic acid. The C-H stretching due to the formation of intermolecular and intramolecular bonds between algae and carboxylic acid groups corresponds to the water-related absorbance. As for the biodegradable analysis, the addition of acetic acid into cinnamon, demonstrated low moisture absorption thus decelerating the degradation due to low swelling rate and microorganism activity. In conclusion, good tensile properties and longer degradation rate are achievable with the addition of 0.3% of acidic acid with 5% of cinnamon blended with the algae matrix.

Keywords: algae; acetic acid; cinnamon; tensile strength; biodegradable.

Introduction Although plastics are a part of our daily lives, they pose threat to the environment. Approximately, 70% of the landfill consists of plastic [1]. Majority of the plastics found in a landfill are single utilize plastics, for example, basic supply plastic packs and plastic containers. These plastics are difficult to decay due to their non-biodegradable property. Some of these non-biodegradable plastics do also end up in the marine ecosystem posing yet another colossal threat to the marine lives. Amidst all this plastic waste ending up in landfills and oceans, finding alternatives to replace plastics is a possible solution. Recently, most bioplastics which have already been produced use biomaterials like corn starch and soybean [1]. Moreover, other food harvests, for example, cassava, wheat, potato, and sago have also been transformed into plastic to supplant oil-based plastic [1]. Since those products are food assets for human, continuous transformation of those yields into plastic will soon interfere with the human sustenance supply by reducing the world's sustenance assets.

Background: In the effort to prevent interference with food assets, other biomaterials were assessed. Algae or seaweed is an environmental asset that exists in boundless amounts which can be cultivated in a naturally inviting way. The Agarose chemical structure provides a good support for films. In addition, it’s reported that the films which are made of algae are transparent, strong, and flexible. Based on a previous study, antimicrobial polymer film was able to restrain microbial development, hence, broadening the time span of usability of sustenance [2]. The carboxylic acid is a good candidate to be applied as a crosslinker. Citric acid can interact with starch to improve the tensile strength, thermal stability and decrease the dissolution of starch films in water and formic acid [3]. However, acetic acid which is also a member of the carboxylic acid family has not been researched on its potential as a crosslinker agent. In addition, cinnamon or cinnamaldehyde which is an organic compound with the formula $C_6H_5CH{=}CHCHO$ occurs naturally as a predominant trans (E) isomer giving cinnamon its flavor and odor [1]. It is a flavonoid that is naturally synthesized by the shikimate pathway [2]. This pale yellow, viscous liquid occurs in the bark of cinnamon trees and other species of the genus Cinnamomum. The essential oil of cinnamon bark contains about 50% cinnamaldehyde [3]. Cinnamaldehyde is also used as a fungicide [17]. Proven effective on over 40 different crops, cinnamaldehyde is typically applied to the root systems of plants [5]. Its low toxicity and well-known properties make it ideal for agriculture. Cinnamaldehyde is an effective insecticide, and its scent is also known to repel animals, such as cats and dogs [17]. It has also been tested as a safe and effective insecticide against mosquito larvae [18]. At a concentration of 29 ppm, cinnamaldehyde can kill half of Aedes aegypti mosquito larvae within 24 hours [19]. Furthermore, the trans-cinnamaldehyde also works as a potent fumigant and practical repellant for adult mosquitos [20]. By adding cinnamon active chemicals in the packaging system [5], the growth rate of microorganisms in food can be inhibited or reduced. Among other antimicrobials, cinnamaldehyde, which is a major component of cinnamon also possesses antimicrobial activity and has been utilized in the processing of milk, chicken, and meat [5,6].

Objectives: The objectives of this study were to (1) assess the suitable percentage loading of acetic acid and algae film, and, to (2) characterize the algae film based on the tensile test, FTIR, SEM and biodegradability test by adding cinnamon powder.

Methodology: The acetic acid was purchased from Sigma Aldrich (Selangor, Malaysia). Commercialized red type algae powder and cinnamon powder were purchased from a local store in Selangor, Malaysia. For the preparation of the first phase film, 2% algae powder, 1.5ml glycerol solution, 0.1% acetic acid and 1% cinnamon powder were weighed individually using electronic mass balance. Chemicals such as Ca(OH)2 was obtained in palette form. Ca(OH)2 was dissolved in distilled water to form a concentration of 0.2% (w/v). Acetic acid, however, was obtained in liquid form. It was diluted to 0.2% (w/v) using distilled water. The algae, glycerol, acetic acid and distilled water were mixed in a beaker which was then heated up to 90˚C on a hot plate and held at that temperature for 25 minutes. The stirring speed of the magnetic stirrer was set at a constant speed of at 250 rpm, to avoid the formation of bubbles and to maintain the homogeneity of the solution. Then, the mixed solution was cooled down to 65˚C for 35 min. During cooling, stirring was continued to prevent the formation of bubbles and solidification of the solution. The second phase of the film begins with the addition of the cinnamon. The procedure is as the same as preparing the film in the first phase. An algae blend films were prepared using solution casting method. Pure algae films (0% acetic acid) were set up as a control sample. The solution was cast into a square form (18 x 27cm) of the acrylic plate. Upon casting, drying process took place in an oven at a temperature of 50˚C for 24 hours.

Material. The film thickness was measured using an electronic gauge (Digitronic Caliper) with accuracy ranging between 0.1% and 1% as a function of thickness value (0-100 µm or 0-1000 µm). Seven replicates were made for each type of film formulation.

Scanning Electron Microscopy (SEM). The surface morphology of the films was studied using Scanning Electron Microscope (SEM) JSM 5600 with magnifications up to 100,000x. Prior to carrying out the observation, the samples were subjected to sputter coating with a layer of carbon using Polaron SC515. This procedure was performed to ensure the samples' morphology can be clearly observed under SEM and to prevent any electrostatic charging during observation.

Tensile test. The ASTM D882-02 machine was used for this test. The load of the machine was set at 5 kN with the speed of 20 mm/min. 7 replicates of strips for each composition were cut at the dimensions of 70 mm x 10 mm. The result of the tensile strength and elongation at break can be assessed through the graph of the stress-strain curve.

Fourier Transform Infrared Spectrometry (FTIR). FTIR Spectroscopy (Perkin Elmer System spectrum 100; PerkinElmer, United States) was used to observe and study the functional groups on the sample. The resolution was set up on 4cm-1 in a spectral range of 4000 to 600 cm-1 and 32 scans per sample. Different peaks (various functional groups of chemical elements) of the IR spectrum were observed along the selected initial angle to the final angle.

Soil burial test. Degradation behavior of algae-based film acetic acid and cinnamon powder was tested by soil burial test. The films dimension of 20 mm x 20 mm were cut and weighed and five replicates were made for each formulation. Each sample was buried in depth of 50mm in various spots loaded with composted soil. The burial plan was set on the open space and based on the normal climate change. The degradation rate of the samples were controlled by recording change in weight for every seven days for six months.

Findings: Effect of film thickness

Algae-based film with acetic acid. In food packaging application, thickness is a crucial aspect which requires specific attention from the material design. The thickness of the packaging will highly influence other important properties such as the strength, elasticity and moisture content. The main purpose of effective food packaging is to secure the food from food pathogens thus extending its shelf life. This will ensure the quality of the food and its nutrient to be intact. The general thickness of films for food packaging is less than 0.3 mm (13). Table 1 demonstrates that the fabricated film from solution casting method produces the same thickness with different loading of acetic acid.

Table 1: Effect of addition of acetic acid on thickness of algae-based film

Sample (% acetic acid)	Thickness (mm)
0	0.2 ± 0.01
0.1	0.2 ± 0.01
0.3	0.2 ± 0.01
0.5	0.2 ± 0.02

Algae-based film with the addition of cinnamon powder. In contrast, a variation of cinnamon powder loading in the algae-based film resulted in different thickness (Table 2). The thickness of the film increased linearly with the increase of cinnamon powder which may have resulted from the increased content of non-solvent matters in the film-forming mixture [6]. This trend was similar to the previous study by Bahram et al. (2012), where whey and pectin protein were incorporated into cinnamon.

Table 2: Effect of addition of cinnamon powder on thickness of algae-based film

Sample (% cinnamon)	**Thickness (mm)**
0	0.2 ± 0.01
1	0.3 ± 0.00
3	0.43 ± 0.07
5	0.51 ± 0.04

Tensile test.

Algae Film with Acetic Acid. Tensile strength, elongation at break, and modulus elasticity of algae-based films were affected by the different percentages of acetic acid and cinnamon are demonstrated in Figure 1, 2, and 3, respectively. The displayed results are an average of seven replicates for each formulation. Formulation without the acetic acid (control) measured the highest tensile strength up to 4.9 MPa. Addition of acetic acid in the algae film decreases the tensile strength to half compared to the control film, probably due to the different molecular structure of the acetic acid even though it comes from the same carboxylic acid family [8]. On the other hand, the elongation at break, also known as fracture strain, is the ratio between changed length and initial length after breakage of the test film. It expresses the capability of the sample to resist changes in shape without crack formation. Figure 2 demonstrated that the effect of acetic acid on elongation at break gradually increased with the increasing percentage of acetic acid by up to 0.5%. Highest elongation at break was measured with 0.5% acetic acid at 27.34% Eb, while, the lowest elongation at break was shown by 0% acetic acid at 20.14% Eb. Figure 3 illustrated an increase in modulus of algae-based films similar to the tensile strength with an increase in the concentration of acetic acid. Based on Figure 3, the lowest elastic property was recorded for 0.5% acetic acid. This finding was similar to previous attempts to increase the percentage of acetic acid by up to 1% [3]. The film obtained was transparent but could easily tear off when folded. Therefore, the addition of acetic acid neither improves the tensile strength nor the elongation at break.

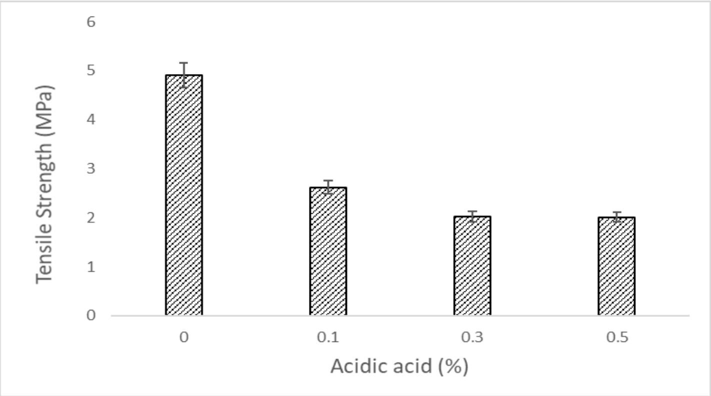


Figure 1. Effect of acetic acid on tensile strength of algae-based film

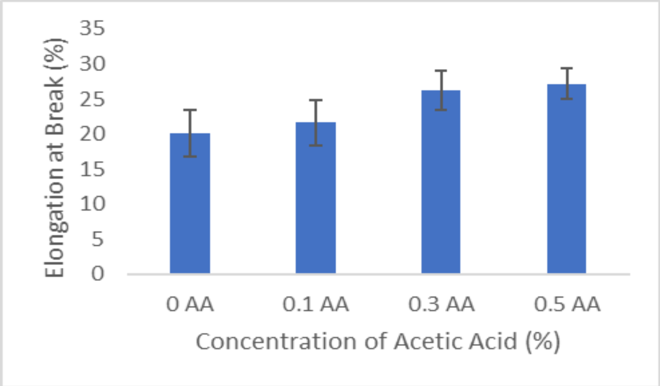


Figure 2. Effect of acetic acid on elongation at break of algae-based film

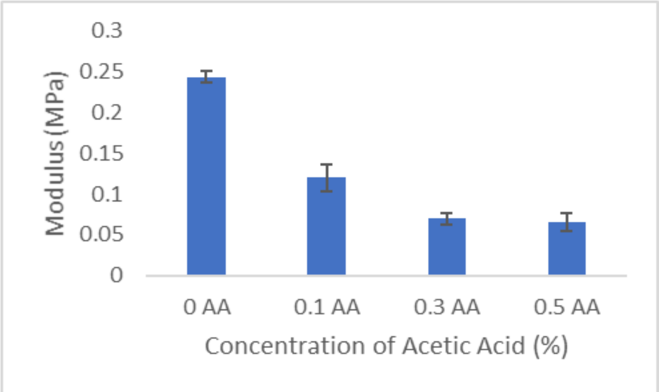


Figure 3. Effect of acetic acid on modulus strength of algae-based film

Based on the observation, the film without the acetic acid possessed better tensile strength and modulus strength compared to the algae film with the acetic acid, whereas, the elongation at break was better in algae film with the acetic acid than a film without acetic acid, which is considered as an important factor in this study. Algae Film with Acetic Acid and cinnamon. Due to good tensile and modulus strength and acceptable flexibility as shown in the elongation at break results in study of algae with acetic acid, 0.3% acetic acid content was selected to be used with the cinnamon powder. This percentage was selected to be used for further investigation alongside the addition of different ranges of cinnamon powder (phase two). The percentages of cinnamon powder tested were 1, 3 and

5%. The control sample contained 5% cinnamon without the addition of acetic acid. Figure 4 demonstrates the tensile strength of algae film which increases with increasing percentages of cinnamon. The control sample without acetic acid possessed the least tensile strength. Meanwhile, the maximum tensile strength was achieved with 5% cinnamon due to the good intermolecular interaction between agar and cinnamon molecules. This finding was also supported by a previous study [10] which also recorded a similar pattern where tensile strength increased with the addition of cinnamon bark oil into the alginate film.

However, in this study, the highest cinnamon loading did affect the elongation at break compared to the other two percentages. Figure 5 indicated that the 3% cinnamon loading has a good elongation at break compared to the 5% cinnamon loading. Based on the current findings, 3% cinnamon with 0.3% acetic acid yielded a good and accepted elongation at break of the algae film. This was made possible with the right amount of acetic acid in strengthening and adhering to the intermolecular bonds between the agar and cinnamon molecules.

Hence, the addition of acetic acid into algae-based film clearly indicated that the acetic acid molecules affect the adjacent molecules by increasing the distance and reducing the internal force resulting in a more flexible film. The interference with adjacent molecules affects the intermolecular and intramolecular linkage of the polymer thus strengthening the structure of the algae film [9]. Based on Figure 6, the modulus elasticity of the algae film was found to display the same trend as the tensile strength results. The addition of 5% cinnamon was found to increase the stiffness of the algae film up to 0.323 MPa, compared to films with 1% and 3% cinnamon which recorded a modulus strength of 0.152 MPa and 0.183MPa, respectively.

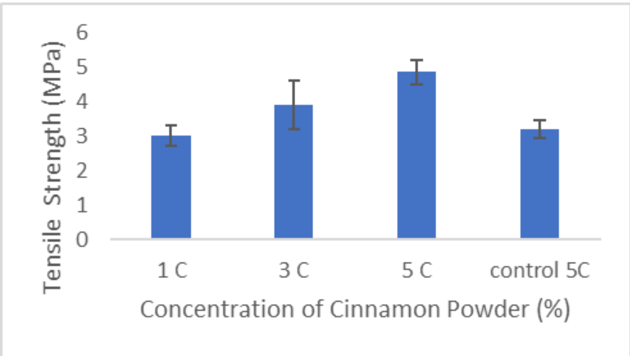


Figure 4. Effect of cinnamon powder on tensile strength of algae-based film

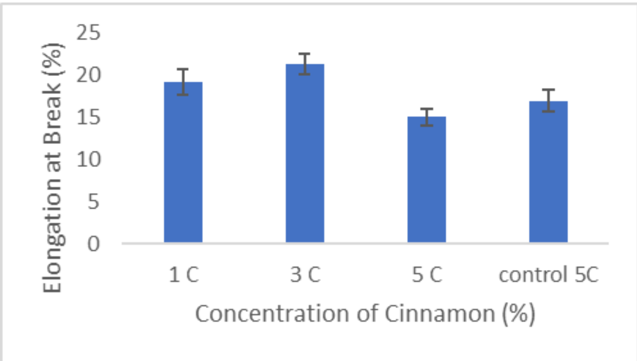


Figure 5. Effect of cinnamon powder on elongation at break of algae-based film

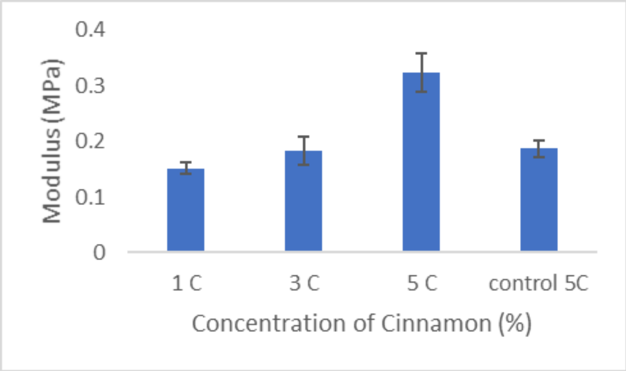


Figure 6. Effect of cinnamon powder on modulus elasticity of algae-based film

Scanning electron microscopy. Figure 7 shows the microstructure of agar and cinnamon powder which both having irregular shape and size approximately 50 – 100 µm. The micrograph of agar powder observed under SEM at magnification of X300. Based on Figure 1**,** it can be seen that the particle shape of algae powder is irregular and different from each other. This finding is quite similar with previous study by Nowak & Lisowska-Oleksiak (2014)**.**

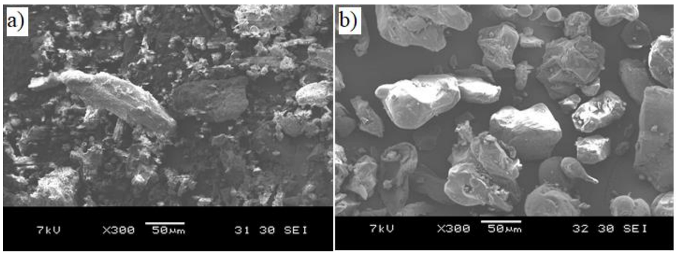


Figure 7. SEM microstructure of a) agar and b) cinnamon powder at X300 magnification

Based on the observation, it was found that the film with the addition of the acetic acid film showed more homogeneity than the control sample (Figure 8(a)). The addition of acetic acid into the blend may help the infusion of glycerol into the algae molecular structure by accelerating the disintegration and suspension of algae sediment. By comparing the acetic acid loading in the film, Figure 8 (b) with 0.1% content of acetic acid shows the most homogenous phase and having the least roughness.

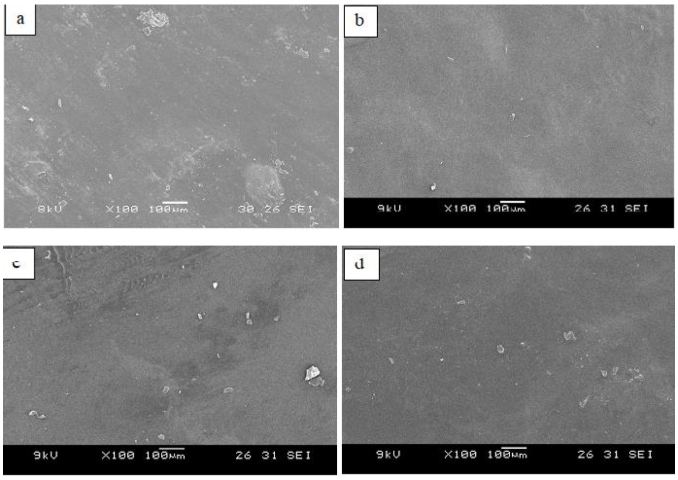


Figure 8. SEM micrographs of 2% algae-glycerol film with (a) 0%, (b) 0.1%, (c) 0.3%, and (d) 0.5% of acetic acid content at X100 magnification

The agglomeration of cinnamon powder makes the surface become rough coupled with high waviness. Voids were observed for all the percentage of cinnamon loading but Figure 9(a) with 1% of cinnamon loading having the high percentage of void. Several mini voids and thin cracks also observed on the 3% cinnamon (Figure 9(b)). This probably happens because of the cinnamon powder were not homogenously dispersed throughout the film sample thus caused insufficient filler. According to the previous researchers, the filler concentration did affects the interparticle distance [7]. At low filler concentration, the interparticle distance is huge thus the mechanical strength hardly be improved. Figure 9(c) shows the morphology of 5% cinnamon powder loading with acetic acid shows an absence of separating phase between the agar and cinnamon blend and these results are in agreement with Maitra & Shukla, (2014) research where higher volume of filler generally could avoid the phase separation to occur and correlate with the tensile test result. However, the control sample with 5% cinnamon powder without addition of acetic acid (Figure 9(d)) have hairline crack due to the brittleness effect. From this analysis, it found that 5% cinnamon with addition of acetic acid is the sufficient amount to enhanced the interaction between the algae matrix and the cinnamon filler.

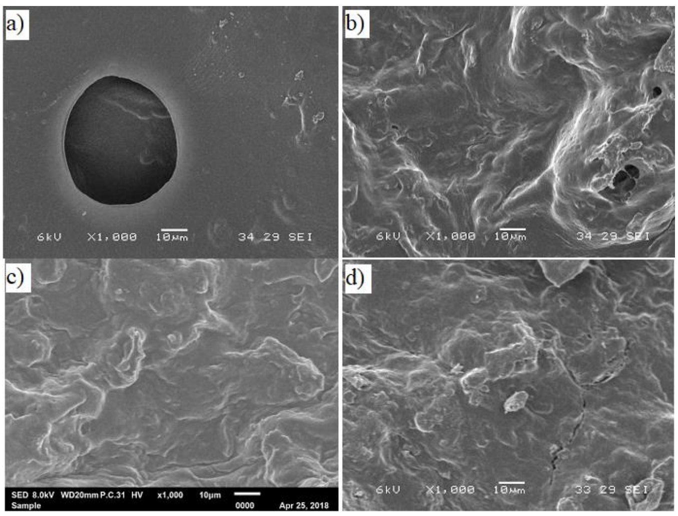


Figure 9. SEM micrograph of algae-glycerol with 0.1% acetic acid with (a) 1%, (b) 3%, (c) 5% of cinnamon loading, (d) 5% cinnamon loading without acetic acid at X1000 magnification

Fourier Transform Infra-Red (FTIR) Spectroscopy. The characteristic peaks of agar are at 1013 cm and 931 cm^{-1} in Figure 10 indicated C-O stretching group of 3,6 anhydrogalactose [11]. As for cinnamon the specific absorbance band at 1635 cm^{-1} revealed the stretching vibration of C=O bond for cinnamaldehyde [12].

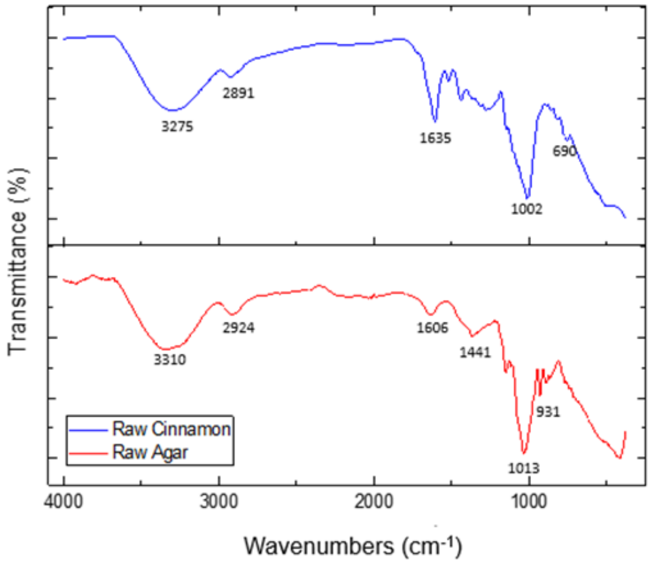


Figure 10. FTIR spectra of cinnamon powder (top) and agar powder (below)

Figure 10 shows the FTIR spectra of cinnamon powder and agar powder, respectively. The peak at 1700 - 1720 cm^{-1} indicated a stretching vibration of the carbonyl group in carboxylic acid (acetic acid). This should be presented if there was any chemical linkage between acetic acid and glycerol from acid groups esters, and also between agar and acetic acid from esters [13]. Since there is no peak observed between 1700 - 1720 cm^{-1} in Figure 9, it can be said the crosslinking linkage is not formed when acetic acid is added into the algae-based blends due to no chemical reaction.

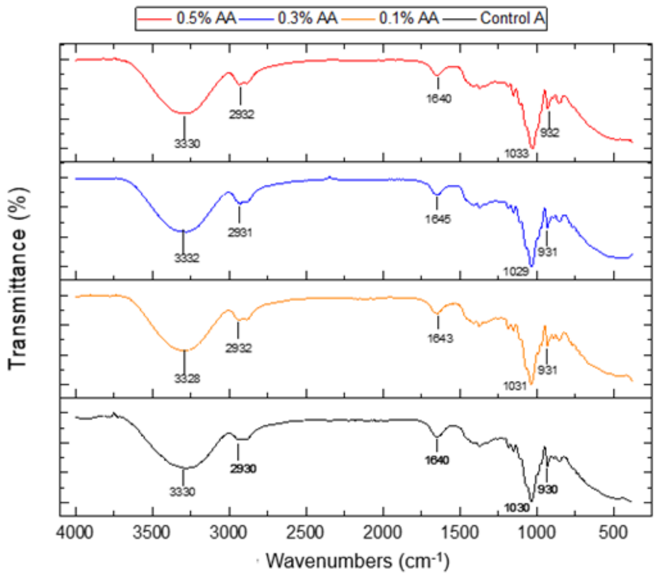


Figure 11. FTIR spectra of algae-based film crosslinked with different acetic acid loading

Figure 11 shows the FT-IR spectrum of three different blends algae-based film. 5% of cinnamon powder without acetic acid, 0.1% acetic acid without cinnamon powder, and 0.1% acetic acid with 5% cinnamon powder respectively. The obvious band between these three is at 1716 cm^{-1} on crosslinked acetic acid 5% cinnamon film which it associated to C=O and attributed to the carboxyl and ester carbonyl bands where there was chemical linkage between acetic acid and agar from acid groups esters, and also between cinnamon and acetic acid from esters [8]. With that, this suggesting that crosslinking was developed indicated the present of crosslinker proven by the higher tensile strength (Figure 12). The hydroxyl group's band around 3328 cm^{-1} become less intense when cinnamon is added into the formulation. Based on the analysis above, it showed that lower O-H bond in the algae will make the polymer become more hydrophobic. This assumption is tally with the water absorption test where algae-film incorporated with cinnamon powder gives lower water uptake than algae-film incorporated without cinnamon powder.

By comparing the specific absorbance band at 1635 cm^{-1} which represented the stretching vibration of C=O bond for cinnamaldehyde [12], 5% cinnamon film without acetic acid have less intense peak compared to 5% cinnamon with acetic acid. The diminishing band of cinnamaldehyde promoting lesser antimicrobial activity of the film as proven by the highest weight loss of 5% cinnamon film with acetic acid after buried in soil for 28 days.

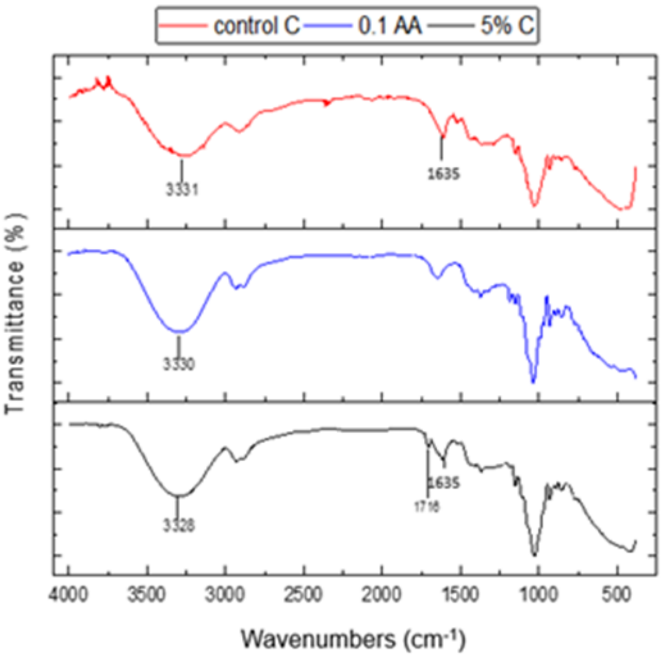
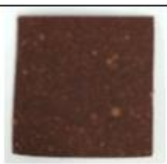
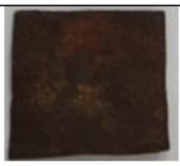
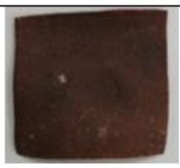
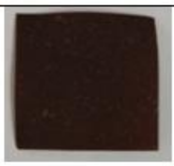


Figure 12. FTIR spectra of algae-based film of different formulation

Table 1. Macroscopic appearance of algae-based films with acetic acid after being buried for 28 days

Film (% acetic acid)	0 days	14 days	28 days
0 (control sample)			
0.1			
0.3			
0.5			

Table 2. Macroscopic appearance of algae-based films with cinnamon and 0.3% acetic acid after being buried for 28 days

Film (cinnamon)	0 days	14 days	28 days
Control sample			
1			
3			
5			

The gradual biodegradation is observed in the pictorial of film's surface degradation shown in Table 1 and 2**.** The samples were place outdoor and exposed to the actual weather. The excess water permeated through the soil and diffused into the samples under rainy conditions, hence causing swelling and films become soften. It also can be noticed that the color of all the films are turning into darker especially film with cinnamon loading. Changing into darker color is a sign of biodegradation occur on the film (Gautam & Kaur, 2013). Based on the observation, up to 14 days, most of the films still retained their shape except for 0.5% acetic acid with 5% cinnamon. This may due to high acetic acid and cinnamon content, respectively. At the end of the period, all sample barely retained their shape and start to tear apart and wrinkle. 1% cinnamon shows the most drastic physical changes. It is understood before that cinnamon is antimicrobial agent by its cinnamaldehyde functional group and the aroma of the cinnamon powder may not attract the microorganisms like ants, worms and even microorganism thus decelerate the degradation rate of the film. This study is in accordance with the previous research, as seen in the Table 2, the lower the cinnamon percentage, the rapid the composability rate. Even though different state of cinnamon used in this study resulting different biodegradation behavior. When considering the high-water absorption and low intensity of cinnamaldehyde of the cinnamon percentages, the appearance changes of the film is acceptable.

However, the sample in this research prone to behaved like Zhang *et al*. (2015) study where the alginate film incorporated with cinnamon bark oil showed less biodegradation compared to the alginate film without cinnamon bark oil. It can say that the addition 0.3% of the acidic acid plays an important role in order to reduce the rate of decomposition of the algae film with the cinnamon as compared to the control sample of 5% cinnamon without acidic acid content. Table 3 shows the micrograph from SEM analysis of algae-based films before and after soil burial at magnification of X100. Overall, surface of all samples become rougher and number of pores became higher after 28 days of soil buried test. This confirms the samples underwent biodegradation phase. By the observation in Table 3, the highest number of pores are obviously started with the control sample, followed by the 0.1%, 0.3% and 0.5%, respectively. As for cinnamon cooperated films as seen in the Table 4, the agglomeration of cinnamon become more obvious as the film swell thus underwent rapid degradation. The most degrade sample can be seen on the cinnamon controls sample without acidic acid which pores are present nearly at the all spots and the surface swell the most. The sample of 1% cinnamon with 0.3% acetic acid show lesser pore percentages as compared to the control sample. Increasing amount of cinnamon powder tend to reduce the degradation rate because of the hydrophobicity of the acetic acid and the aromas of the cinnamon itself may block and not attract the attention of insects and microorganism to attack the algae film (Kwak *et al*., 2017).

Table 3. SEM micrograph of algae-based films with acetic acid before and after soil burial at X100 magnification

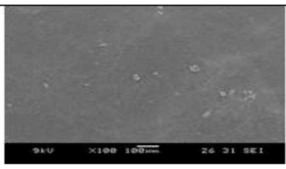
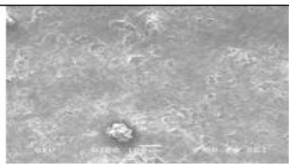
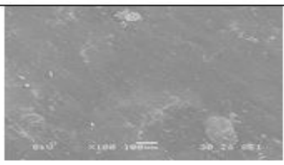
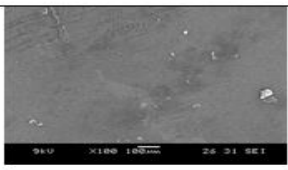
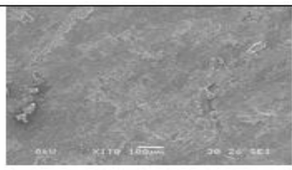
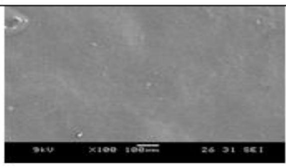
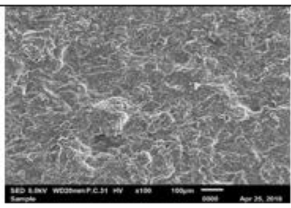
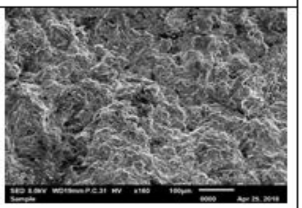
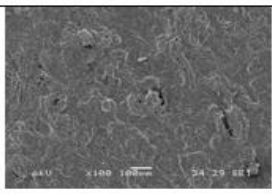
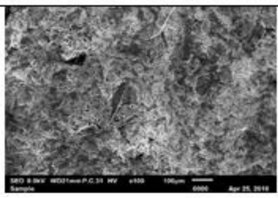
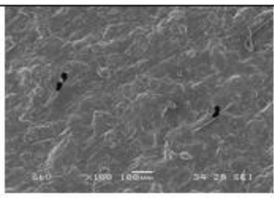
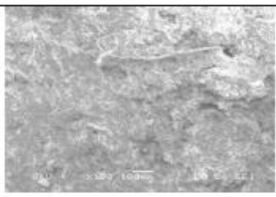
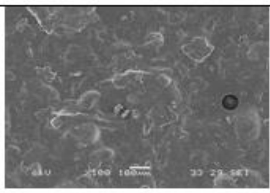
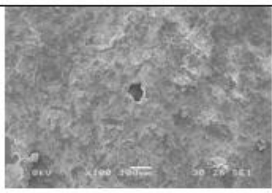
Film (% acetic acid)	Before soil burial	After soil burial
Control sample		
0.1		
0.3		
0.5		

Table 4. SEM micrograph of algae-based films before and after soil burial at X100 magnification

Film (% cinnamon)	Before soil burial	After soil burial
Control sample		
1		
3		
5		

Conclusion

The tensile test of 0.3% acetic acid algae-based film showed good enhancement after incorporated with 5% cinnamon powder. The film achieved tensile strength at 4.8 MPa and elongation at 15%. From SEM morphology analysis found that the higher the amount of cinnamon, provide good adhesion and mechanical interlocking between the algae and the cinnamon with the existent of acidic acid hence lead to reduce the degradation rate of the film. The film demonstrated a continuous phase and exhibited the characteristic band at 1716 cm^{-1} studied by FTIR analysis. It can be concluded that, with the suitable percentage of the cinnamon and the acidic acid would tremendously affect the tensile behavior and biodegradation rate of the algae film.

Output: 8 Journals scopus indexed

Future Plan of the research: This Preliminary research succeed to develop a biopolymer film by using the natural based algae without compromising the food chain supplies. Moreover, this biofilm system promoting the nontoxic and environmetal friendly decomposition waste materials to landfill. Would like to focus more on the enhancement of the prolongation decomposition rate of the biopolymer. In order to achieve the main objective, We would like to focus more on below signicant studies:

1. Study the suitable antioxidant and antimicrobial for the biopolymer.
2. Determine significant treatment to the biopolymer, such as plasma treatment and/or vacuum treatment in order to prolong the shelf-life of the bioplastic film especially when in contact with the food content.
3. Evaluate the suitable additive to enhance the elongation at break/stiffness rate of the bioplastic.

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